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# Duodenal-Jejunal Bypass and Jejunectomy Improve Insulin Sensitivity in Goto-Kakizaki Diabetic Rats Without Changes in Incretins or Insulin Secretion

Gastric bypass surgery can dramatically improve type 2 diabetes. It has been hypothesized that by excluding duodenum and jejunum from nutrient transit, this procedure may reduce putative signals from the proximal intestine that negatively influence insulin sensitivity ( $S_I$ ). To test this hypothesis, resection or bypass of different intestinal segments were performed in diabetic Goto-Kakizaki and Wistar rats. Rats were randomly assigned to five groups: duodenal-jejunal bypass (DJB), jejunal resection (jejunectomy), ileal resection (ileectomy), pair-fed sham-operated, and nonoperated controls. Oral glucose tolerance test was performed within 2 weeks after surgery. Baseline and poststimulation levels of glucose, insulin, glucagon-like peptide 1 (GLP-1), and glucose-dependent insulintropic polypeptide (GIP) were measured. Minimal model analysis was used to assess  $S_I$ .  $S_I$  improved after DJB ( $S_I = 1.14 \pm 0.32 \times 10^{-4} \text{ min}^{-1} \cdot \text{pM}^{-1}$ ) and jejunectomy ( $S_I = 0.80 \pm 0.14 \times 10^{-4} \text{ min}^{-1} \cdot \text{pM}^{-1}$ ), but not after ileectomy or sham operation/pair feeding in diabetic rats. Both DJB and jejunal

resection normalized  $S_I$  in diabetic rats as shown by  $S_I$  levels equivalent to those of Wistar rats ( $S_I = 1.01 \pm 0.06 \times 10^{-4} \text{ min}^{-1} \cdot \text{pM}^{-1}$ ;  $P = \text{NS}$ ). Glucose effectiveness did not change after operations in any group. While ileectomy increased plasma GIP levels, no changes in GIP or GLP-1 were observed after DJB and jejunectomy. These findings support the hypothesis that anatomic alterations of the proximal small bowel may reduce factors associated with negative influence on  $S_I$ , therefore contributing to the control of diabetes after gastric bypass surgery.

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Gastric bypass surgery results in rapid and sustained remission of type 2 diabetes (1,2). Experimental evidence from both animal (3,4) and human studies (5–7) suggest that the improvement of diabetes after gastric bypass is partly independent of weight loss and decreased caloric intake. Possible weight-independent mechanisms include changes in gut hormones (8), bile acid metabolism (9), nutrient sensing (10), and microbiota (11).

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Roux-en Y gastric bypass surgery (RYGB) creates a complex rearrangement of gastrointestinal anatomy, involving reduction of the volume of the stomach, bypass of proximal segments of the small bowel (duodenum and jejunum), and expedited delivery of nutrients to the ileum. Such rearrangement of the gastrointestinal anatomy is likely to activate more than one mechanism of action (12). Elucidating the specific role of distinct anatomic alterations imposed by gastric bypass has far-reaching implications. This knowledge may simplify the design of future surgical or device-based approaches, inform pharmaceutical research regarding new targets for effective antidiabetes drugs, and possibly help elucidate the elusive pathophysiology of type 2 diabetes and other insulin-resistant states.

The substantial weight loss and improvement of diabetes after vertical sleeve gastrectomy (12), a procedure that does not involve rerouting of the small intestine, support a role of gastric manipulations in the mechanisms of improved metabolism after bariatric surgery. However, clinical and experimental data from studies of stomach-sparing procedures, such as duodenal-jejunal bypass (DJB) (3,4,10,13–15), endoluminal duodenal sleeve (16,17), ileal-interposition (18,19), jejuno-ileal bypass (20), and jejunectomy (21), show that these approaches can improve type 2 diabetes with relatively minor changes in body weight (22).

Two theories have been proposed to explain how small bowel manipulations can improve glucose metabolism: the distal (or hindgut) hypothesis suggests that diabetes control results from the rapid delivery of nutrient chyme to the distal intestine, enhancing physiologic signals that improve glucose metabolism (i.e., increased incretins, specifically glucagon-like peptide 1 [GLP-1], or increased intestinal uptake of glucose) (12,18). The proximal (or foregut) hypothesis suggests that the effect of gastric bypass on glucose metabolism depends on the exclusion of duodenum and proximal jejunum from the transit of nutrients, possibly preventing the secretion of a putative signal that promotes insulin resistance and type 2 diabetes (12,23–25).

In this study, we sought to investigate the role of the proximal and distal gut on glucose homeostasis by

measuring insulin sensitivity ( $S_I$ ) and incretin (GLP-1 and glucose-dependent insulintropic polypeptide [GIP]) levels early after bypass or resection of different segments of the small intestine.

## RESEARCH DESIGN AND METHODS

### Animals

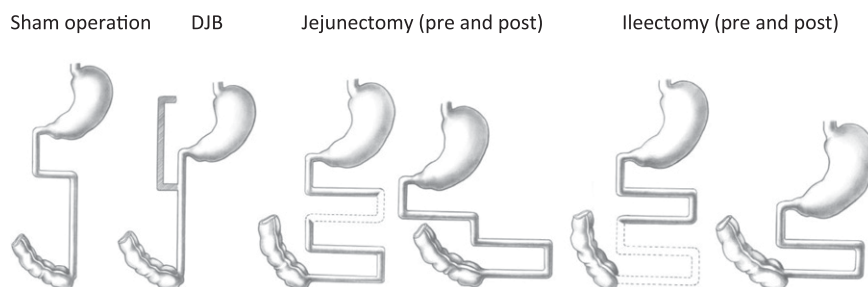
A total of 100 male rats aged 10 weeks were used for this study. Fifty Wistar (normal) and 50 diabetic Goto-Kakizaki (GK) rats were housed individually in hanging wire cages. Wistar rats were bred in-house while GK rats were purchased from Taconic M&B A/S (Tombjerg, Denmark). Animals were housed in a controlled room at 22°C with a 12-h day/night cycle (lights on at 07:00 and lights off at 19:00). Rats were allowed to adapt to their new environment for 4 days before the surgery. Both Wistar and GK animals randomly underwent DJB, jejunal resection (jejunectomy), ileal resection (ileectomy), or a sham operation. Ten animals were originally assigned to each group. Twenty animals, 10 Wistar and 10 GK rats, were used as controls. Survival rates after surgery were 90% for sham operations in both strains, 80% for jejunectomy and ileectomy in both strains, 70% for DJB in Wistar rats, and 60% for DJB in diabetic animals. All experimental procedures were approved by the Catholic University of Rome Institutional Animal Care Committee.

### Interventions

The rats were anesthetized using ketamine (75 mg/kg intramuscularly) and xylazine (10 mg/kg intramuscularly). Ten milliliters of sterile 0.9% NaCl was administered s.c. prior to surgery. Figure 1 summarizes all of the interventions performed.

### DJB

DJB is a stomach-sparing modification of RYGB. Similarly to RYGB, the DJB involves a bypass of the entire duodenum and the proximal jejunum; the stomach anatomy, however, is left unperturbed. The procedure was performed according to the original technique reported by Rubino and Marescaux and Rubino et al. (3,4). In brief, the duodenum was divided just distal to



**Figure 1**—Sketch of the surgical interventions. From left to right: sham, DJB, pre- and postjejunectomy, and pre- and postileectomy.

the pylorus, and its proximal end was closed using absorbable suture material. Next, the jejunum was transected ~10 cm from the ligament of Treitz. The distal cut end of the jejunum was reconnected to the stomach through an end-to-end gastrojejunostomy. The proximal cut end (duodenal-jejunal limb) was reconnected to the jejunum at a distance of 15 cm downstream of the gastrojejunostomy in an end-to-side fashion. The resulting anatomy includes the following intestinal segments: 1) the biliopancreatic limb, consisting of the duodenum and the proximal 10 cm of jejunum; this limb is excluded from nutrients passage but continues to carry bile and pancreatic juice; 2) the alimentary limb (or "Roux limb"), consisting of distal jejunum; this segment is directly connected to the stomach and is exposed to nutrients but not to biliopancreatic secretions; and 3) the common channel, consisting of the entire length of the ileum; this is the only segment where nutrients and biliopancreatic juices mix after DJB.

#### **Jejunectomy**

The small intestine was measured from the ligament of Treitz to the ileocecal junction. The proximal 50% of the intestine was resected. Intestinal continuity was restored by direct anastomosis between the duodenum and the remaining ileum.

#### **Ileectomy**

Starting from 1 cm proximal to the ileocecal valve, a segment of 30 cm of distal small bowel (ileum) was measured and resected. Intestinal continuity was restored by direct anastomosis between the jejunum and the remaining ileum.

#### **Sham Operation**

The sham-operated rats had the same anesthesia as described above. A midline laparotomy was performed, and the stomach and intestines were exposed and gently manipulated. The abdominal cavity was kept open for the same amount of time required to perform the DJB procedures in accordance with previous experiments (3,4). Transection of the gastrointestinal tract was performed at all sites at which enterotomies were performed for the DJB; the intestinal limbs were then reanastomosed to allow unaltered progression of food through the bowel.

#### **Postoperative Care**

Postoperative care was identical for all rats as previously described (26). At the end of the surgical procedures, all rats received sterile 0.9% NaCl (10 mL i.p. and 10 mL s.c.) to maintain hydration during healing, since they were not allowed access to drinking water until the next morning. All rats received ketoprofen (5 mg/kg) as analgesics and enrofloxacin (5 mg/kg) as an antibiotic. The rats were then placed on a heated mat until they fully recovered from anesthesia, after which they were

returned to their home cages. The rats in the surgical groups were only allowed to drink purified water for 12 h after surgery. A liquid diet containing 5% glucose and 0.2% KCl was provided for the next 48 h. The rats were then fed with standard chow for the remaining of the study period.

#### **Body Weight and Food Intake**

Body weight and food intake were measured daily for the duration of the study. Wistar and GK sham-operated rats were pair fed to the animals having intestinal resection and DJB in order to control for the effect of reduced calorie intake on  $S_I$ .

#### **Oral Glucose Tolerance Test**

After an overnight fast, blood glucose was measured in conscious rats before (baseline) and at 15, 30, 60, 90, and 120 min after administration of 2 g/kg glucose by oral gavage. Blood was obtained in heparinized and aprotinin-treated tubes (10  $\mu$ L DPP-4 inhibitor/mL of blood; Millipore, St. Charles, MO) from a tail vein at each sampling point to measure insulin and incretins.

#### **Outcome Measures**

Glucose was measured using a glucometer (OneTouch Ultra; LifeScan, Inc., Johnson & Johnson, Milpitas, CA). Plasma insulin was determined using an ultrasensitive rat insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany): intra-assay coefficient of variation (CV) was 2.9%, and interassay CV was 4.8%. Plasma GIP was measured by an enzyme immunosorbent assay kit (Phoenix Pharmaceutical Inc., Burlingame, CA): sensitivity (minimum detectable concentration) 0.44 ng/mL, intra-assay CV 5–10%, and interassay CV <15%. Active (7,36)amide plasma GLP-1 was assessed by an enzyme immunosorbent assay kit (ALPCO Diagnostics, Salem, NH): intra-assay CV 5.36–6.60%, and interassay CV 5.51–18.87%.

#### **Homeostatic Model Assessment of Insulin Resistance**

The homeostatic model assessment of insulin resistance (HOMA-IR) index (27) was calculated as  $\text{HOMA-IR} = (\text{FBG} \times \text{FPI})/405$ , where FBG denotes fasting blood glucose (mg/dL) and FPI is fasting plasma insulin ( $\mu$ U/mL). The factor 405 accounts for measurement units.

#### **Matsuda-DeFronzo Index**

The Matsuda-DeFronzo (composite)  $S_I$  index (CISI) (28), estimated from the oral glucose tolerance test (OGTT) and validated by Carr et al. (29) in the rat was calculated as follows:  $\text{CISI} = 10,000/(\text{FBG} \times \text{FPI} \times \text{MG} \times \text{MI})^{1/2}$ , where MG and MI denote the mean glucose and mean insulin concentrations, respectively, during the course of the OGTT (area under the curve [AUC]/120 min). The factor 10,000 is an arbitrary scaling constant.

#### **Mathematical Model for Measurement of $S_I$**

The glucose minimal model (30) was used to analyze the OGTT data, as it provides a reliable estimate of  $S_I$

that correlates with the euglycemic hyperinsulinemic clamp. The minimal model is currently used to assess  $S_I$  ( $\text{min}^{-1} \cdot \text{pM}^{-1}$ ) and glucose effectiveness ( $S_G$ ;  $\text{min}^{-1}$ ) from the intravenous and OGTT in humans and has been validated in mice (31,32).

Minimal model equations were:

$$\dot{G} = -(S_G + S_I Z)G + S_G G_b + \frac{Ra}{V_G}, \quad G(0) = G_b \quad (1)$$

$$\dot{Z} = p(-Z + I - I_b), \quad Z(0) = 0 \quad (2)$$

with

$$Ra = \frac{a\Delta G + \Delta \dot{G}}{b},$$

where the overdot means  $d/dt$ ,  $G$  is the glucose concentration (basal value  $G_b$ , and  $\Delta G = G - G_b$ ),  $I$  is the insulin concentration (basal value  $I_b$ ),  $Z$  is a variable related to insulin action,  $Ra$  is the rate of appearance of oral glucose in plasma,  $V_G$  the glucose distribution volume, and  $p$  a parameter ( $\text{min}^{-1}$ ) that represents the rate constant of the remote insulin compartment from which insulin acts on glucose disposal.

The model parameters  $S_G$ ,  $S_I$ ,  $p$ ,  $a(bV_G)^{-1}$ , and  $(bV_G)^{-1}$  were estimated by fitting glucose concentration data. Direct estimation of the population parameters was obtained by the NONMEM method (33).

### Data Presentation and Statistics

Data are presented as means  $\pm$  SD unless otherwise indicated. The AUC was calculated using the trapezoidal rule. One-factor ANOVA and the ANOVA for repeated measurements followed by Tukey test were used for intergroup comparisons. The minimal model  $S_I$  was regressed against the Matsuda-DeFronzo CISI to verify the agreement between the two methods.  $P$  values  $< 0.05$  were considered significant.

## RESULTS

All operated animals lost weight (Fig. 2A) and ate less food (Fig. 2B) compared with nonoperated controls; however, no significant differences among the surgical groups (pair-fed sham, ileectomy, DJB, and jejunectomy) were observed.

Table 1 reports the incremental AUCs of glucose, as well as the insulin resistance index HOMA-IR and the Matsuda-DeFronzo CISI. Among normal rats (Wistar), the incremental glucose AUC was significantly higher in the DJB group and lower in the jejunectomy group ( $P < 0.001$ ) compared with sham-operated controls. Ileectomy increased HOMA-IR and decreased CISI versus sham ( $P < 0.001$ ). In diabetic rats (GK), DJB significantly decreased ( $P < 0.05$ ) the incremental glucose AUC compared with sham-operated animals. Both jejunectomy and DJB decreased HOMA-IR ( $P < 0.001$  and  $P < 0.025$ ,

respectively) and increased CISI ( $P < 0.001$ ) compared with sham-operated controls.

The best fit of OGTT plasma glucose levels, obtained by the oral glucose minimal model, is shown in Fig. 3A for Wistar rats and in Fig. 3B for GK rats. Sham operation was associated with an increase in fasting and postprandial plasma glucose levels (FBG and MG values) in both normal and diabetic rats. Animals that underwent jejunectomy showed significantly lower plasma glucose excursion compared with pair-fed sham controls in Wistar rats. A similar effect was induced by DJB in diabetic rats but not in normal rats (Table 1).

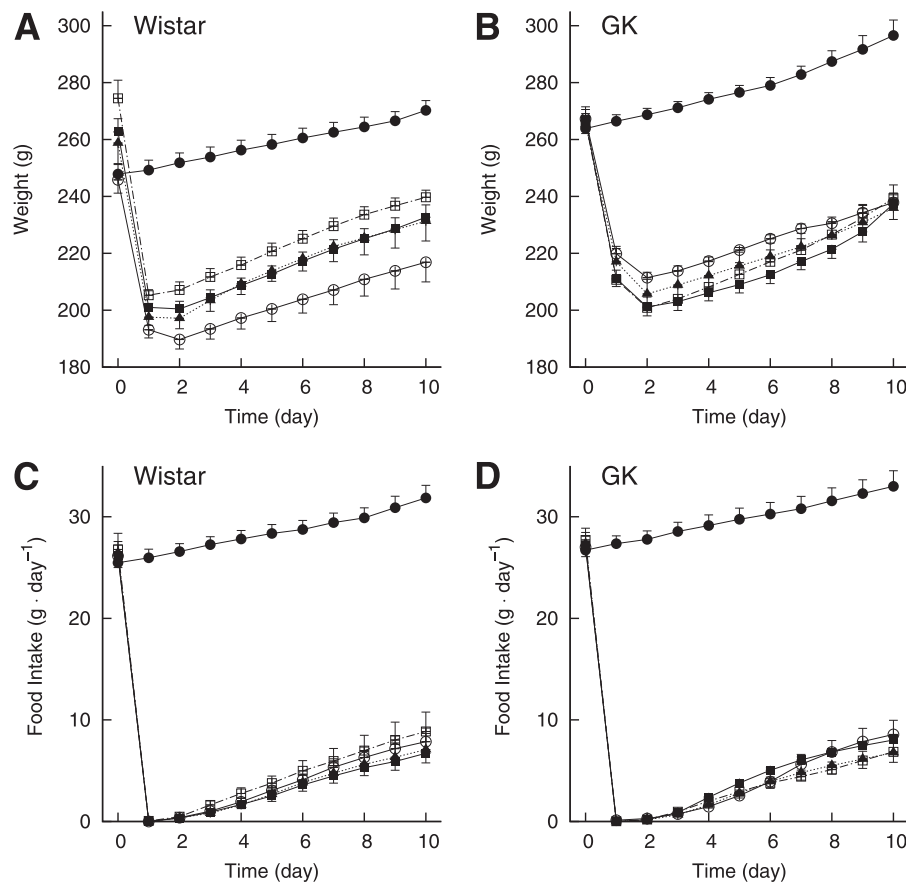
As expected,  $S_I$  was lower in diabetic rats ( $0.40 \pm 0.11 \times 10^{-4}$ ) compared with normal rats ( $1.34 \pm 0.20 \times 10^{-4} \text{ min}^{-1} \cdot \text{pM}^{-1}$ ;  $P < 0.0001$ ) (Table 2). Ten days after surgery, glucose tolerance worsened in sham-operated nondiabetic rats, possibly as an effect of the surgical stress as previously observed in the same rat model (17). Remarkably, DJB ( $P < 0.001$ ) improved  $S_I$  in diabetic GK rats, resulting in levels of  $S_I$  that were similar to those of nondiabetic, sham-operated Wistar rats. A trend for improved  $S_I$  (albeit not statistically significant) was also observed after jejunectomy. The glucose effectiveness was unaffected by the intestinal operations in both GK and Wistar rats (Table 2). To confirm the validity of the minimal model analysis, the  $S_I$  values were regressed against the respective values of the Matsuda-DeFronzo index obtaining  $S_I = 0.55 + 0.036 \times \text{CISI}$  ( $R = 0.69$ ;  $P < 0.0005$ ).

We found no significant difference in the AUC of  $Ra$ , normalized by  $V_G$ , among the different operations (Table 2), suggesting that surgery did not affect glucose absorption. Using the  $V_G$  value of Wistar rats ( $0.22 \text{ L/kg}$ ) estimated by intravenous glucose tolerance test (34), we found that the mean AUC of  $Ra$  per unit weight in our data ( $38.9 \text{ mmol/L} \times 0.22 \text{ L/kg} = 8.56 \text{ mmol/kg}$ ) was 77.8% of the dose ( $11 \text{ mmol/kg}$ ), a percentage close to that observed in humans (30).

Figure 4 shows the time course of the concentrations of insulin and incretins (GLP-1 and GIP) during the OGTT. The AUC for insulin (not reported) was significantly lower in all surgical groups compared with nonoperated control animals but no difference was found among the surgical groups. Ileectomy and jejunectomy induced a greater GIP response compared with sham-operation in Wistar rats. GIP increased only after ileectomy in GK rats.

## DISCUSSION

Exclusion of duodenum and jejunum from food transit and, to a lesser extent, the removal of jejunum improved whole-body  $S_I$  in GK rats, as assessed by the minimal model  $S_I$  and the CISI index.  $S_I$  values obtained by the minimal model were consistent with the CISI values calculated from the experimental OGTT data. The minimal model analysis as a method for evaluating the glucose disposal in rats is well-established in studies (34–37)



**Figure 2**—Time course of body weight in Wistar (A) and GK (B) rats and time course of food intake in Wistar (C) and GK (D) rats before (day 0) and after surgery. Control, black circles; sham, open circles; ileectomy, black triangles; DJB, black squares; and jejunectomy, open squares. Data are shown as means  $\pm$  SE ( $n = 6$ –10 for each group). Significances: weight,  $P < 0.01$  control vs. surgical groups at times from 1–10 days in both Wistar and GK rats; food intake,  $P < 0.001$  control vs. surgical groups at times from 1–10 days in both Wistar and GK rats (symbols omitted in the figure).

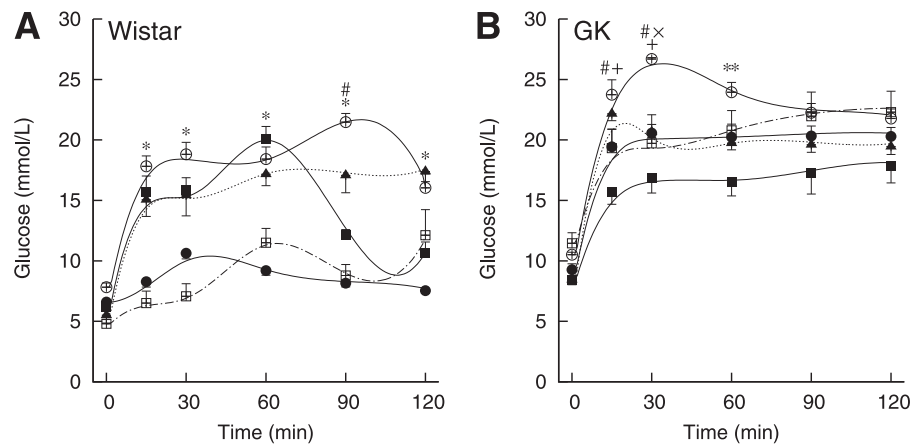
in which  $S_I$  was estimated by the intravenous glucose tolerance test, resulting in values similar to those observed in our investigation. Moreover, hepatic  $S_I$  as calculated by HOMA-IR improved in diabetic rats just a few days after surgery, even in the presence of an increased peripheral insulin resistance due to the surgical trauma.

The current study was specifically designed to investigate the role of distinct anatomic segments of the small intestine in the regulation of  $S_I$ . To minimize confounding influence from weight loss and long periods of improved metabolism,  $S_I$  and glucose tolerance were measured early after surgery.

**Table 1—Incremental AUC of glucose concentration, HOMA-IR index, and CISI**

Indexes	Control	Sham	Ileectomy	DJB	Jejunectomy
<b>Wistar rats</b>					
$\Delta\text{AUC(G)} \times 10^{-2}$ (mmol/L $\cdot$ min)	$2.54 \pm 0.72^*$	$12.47 \pm 1.07$	$12.41 \pm 2.24$	$17.65 \pm 2.06^*$	$6.33 \pm 1.97^*$
$\text{HOMA-IR} \times 10^2$ (mg $\cdot$ dL $^{-1}$ $\cdot$ $\mu$ U $\cdot$ mL $^{-1}$ )	$1.42 \pm 0.15$	$3.68 \pm 0.43$	$12.88 \pm 3.97^*$	$5.80 \pm 1.06$	$4.32 \pm 0.66$
CISI (mg $\cdot$ dL $^{-1}$ $\cdot$ $\mu$ U $\cdot$ mL $^{-1}$ )	$23.94 \pm 2.32^*$	$12.76 \pm 1.30$	$7.36 \pm 1.51^*$	$10.47 \pm 1.30$	$13.72 \pm 1.19$
<b>GK rats</b>					
$\Delta\text{AUC(G)} \times 10^{-2}$ (mmol/L $\cdot$ min)	$12.60 \pm 1.14$	$14.86 \pm 1.16$	$11.50 \pm 7.73$	$9.47 \pm 3.12\&$	$13.19 \pm 1.40$
$\text{HOMA-IR} \times 10^2$ (mg $\cdot$ dL $^{-1}$ $\cdot$ $\mu$ U $\cdot$ mL $^{-1}$ )	$7.37 \pm 1.41^*$	$30.87 \pm 1.43$	$30.36 \pm 9.84$	$18.39 \pm 2.22\#$	$12.01 \pm 0.64^*$
CISI (mg $\cdot$ dL $^{-1}$ $\cdot$ $\mu$ U $\cdot$ mL $^{-1}$ )	$5.27 \pm 0.60^*$	$2.48 \pm 0.10$	$3.82 \pm 0.49$	$5.83 \pm 0.72^*$	$5.21 \pm 0.25^*$

Data are means  $\pm$  SD.  $\Delta\text{AUC(G)}$ , AUC of glucose concentration. Significant differences by ANOVA and Tukey test between sham and the other groups:  $^*P < 0.001$ ;  $\&P < 0.05$ ;  $\#P < 0.025$ .



**Figure 3**—Time course of blood glucose concentration in Wistar (A) and GK (B) rats. Control, black circles; sham, open circles; ileectomy, black triangles; DJB, black squares; and jejunectomy, open squares. Data are shown as means  $\pm$  SE ( $n = 6$ –10 for each group). The lines represent the optimal fitting obtained by the minimal model with the parameters in Table 2. Control, sham, and DJB, continuous lines; ileectomy, dotted lines; and jejunectomy, dashed-dotted lines. Significance: \* $P < 0.001$  control vs. sham; # $P < 0.001$  DJB vs. sham; + $P < 0.05$  control vs. sham;  $\times P < 0.001$  ileectomy vs. sham; \*\* $P < 0.05$  DJB vs. sham.

Our findings show that both resection and bypass of segments of the proximal small bowel (but not distal small bowel) improve  $S_I$  in GK rats, a nonobese rodent model of type 2 diabetes. This is consistent with the proximal (foregut) hypothesis of diabetes control after gastrointestinal bypass surgery. In the present investigation, the bypass of the duodenum and jejunum or the resection of the jejunum did not change GLP-1 in diabetic GK rats. The GIP response to the oral glucose load was similarly unaffected by these procedures, suggesting that, at least in these animals, the effect of these operations is not mediated by changes in these incretins. These results are consistent with those of at least two recent studies (10,38) showing that GLP-1 levels are not dramatically elevated shortly after DJB. This is in contrast with the evidence that RYGB and sleeve gastrectomy result in rapid and large elevations of GLP-1 in response to oral glucose or mixed-meal tests both in humans and rodents. The discrepancy might be due to

the fact that RYGB and sleeve gastrectomy, unlike DJB, involve significant manipulations of gastric anatomy. In fact, a recent study (38) suggests that changes in GLP-1 response to nutrient stimuli may result from alterations of gastric physiology rather than from intestinal rerouting as previously believed.

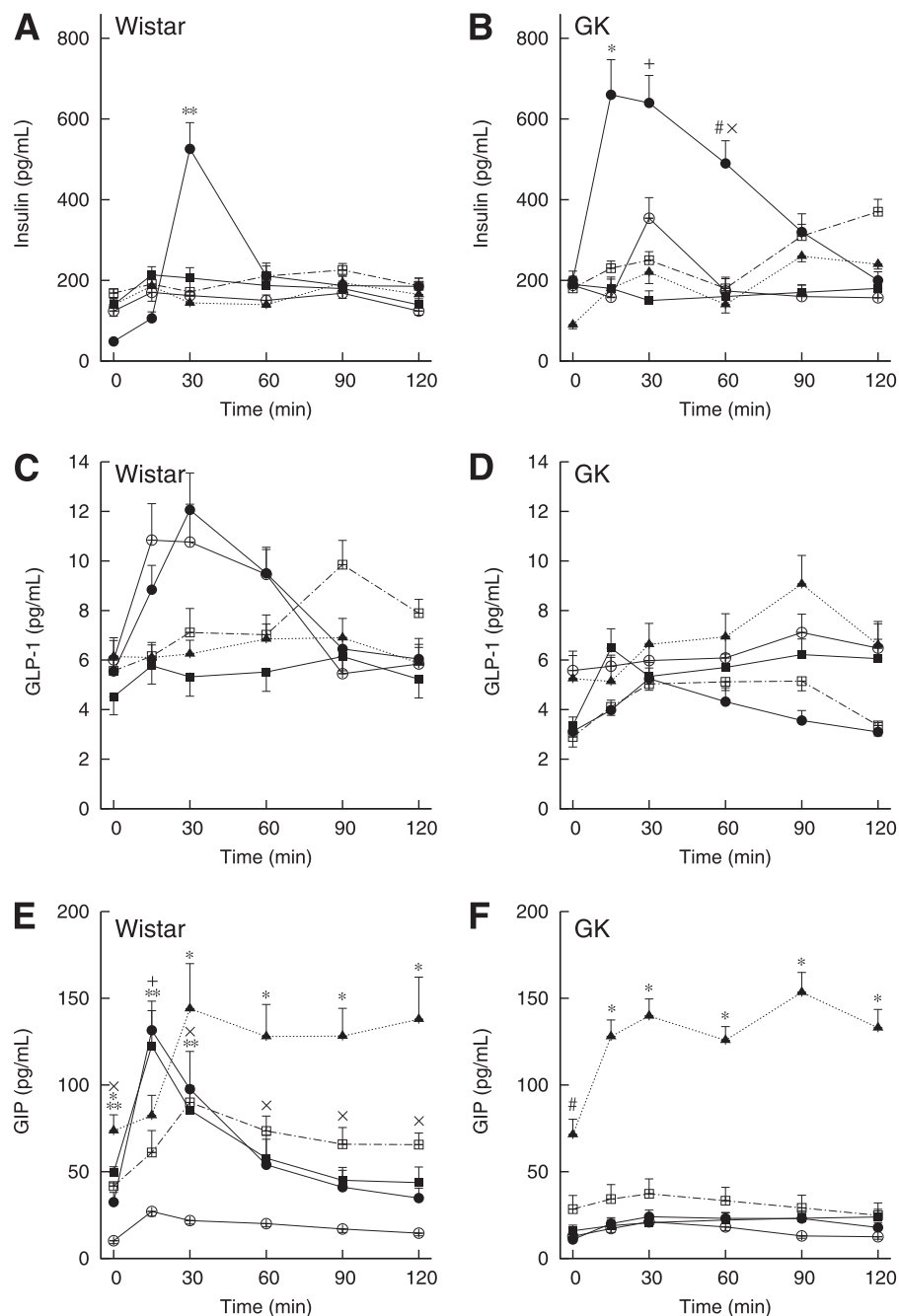
The measurement of GLP-1 levels as early as 10 days postoperatively might also explain why DJB did not affect GLP-1 secretion in the current study. In fact, previous investigations in rodents suggest that elevation of GLP-1 levels may be a late phenomenon after DJB (13–15,39,40).

The rapid improvement of glucose homeostasis in absence of substantial changes in GLP-1 secretion following DJB and jejunectomy in diabetic GK rats suggests that mechanisms other than incretins may play a role in the remission of diabetes after bariatric surgery. Other recent investigations have similarly called into question the role of GLP-1 in the improvement of glucose

**Table 2**—Estimates of minimal model parameters and AUC of the ratio  $Ra/V_G$

Parameters	Control	Sham	Ileectomy	DJB	Jejunectomy
<b>Wistar rats</b>					
$S_G \times 10^2$ ( $\text{min}^{-1}$ )	$3.53 \pm 0.18$	$3.58 \pm 1.07$	$4.04 \pm 0.64$	$3.66 \pm 0.43$	$4.02 \pm 0.86$
$S_I \times 10^4$ ( $\text{min}^{-1} \cdot \text{pM}^{-1}$ )	$1.34 \pm 0.20^*$	$1.01 \pm 0.06$	$0.84 \pm 0.09$	$1.21 \pm 0.18$	$1.03 \pm 0.17$
$p \times 10^2$ ( $\text{min}^{-1}$ )	$3.67 \pm 2.12$	$3.23 \pm 0.61$	$3.26 \pm 0.01$	$3.14 \pm 0.51$	$3.26 \pm 0.32$
AUC ( $Ra/V_G$ ) $\times 10^{-1}$ (mmol/L)	$3.04 \pm 0.55$	$3.58 \pm 0.77$	$3.48 \pm 0.88$	$3.94 \pm 0.37$	$3.49 \pm 0.37$
<b>GK rats</b>					
$S_G \times 10^2$ ( $\text{min}^{-1}$ )	$3.32 \pm 0.75$	$3.64 \pm 0.33$	$3.29 \pm 1.01$	$3.66 \pm 0.31$	$3.65 \pm 0.36$
$S_I \times 10^4$ ( $\text{min}^{-1} \cdot \text{pM}^{-1}$ )	$0.40 \pm 0.11$	$0.60 \pm 0.16$	$0.53 \pm 0.21$	$1.14 \pm 0.32^\S$	$0.80 \pm 0.14$
$p \times 10^2$ ( $\text{min}^{-1}$ )	$2.68 \pm 1.05$	$2.61 \pm 0.85$	$3.11 \pm 0.58$	$3.92 \pm 1.20$	$3.09 \pm 0.21$
AUC ( $Ra/V_G$ ) $\times 10^{-1}$ (mmol/L)	$3.83 \pm 0.31$	$4.25 \pm 0.32$	$4.36 \pm 1.68$	$5.10 \pm 0.66$	$3.76 \pm 0.81$

Data are means  $\pm$  SD. Significant differences by ANOVA and Tukey test between sham and the other groups: \* $P < 0.01$ ;  $^\S P < 0.001$ .



**Figure 4**—Time course of plasma insulin concentration in Wistar (A) and GK (B) rats, active GLP-1 concentration in Wistar (C) and GK (D) rats, and GIP concentration in Wistar (E) and GK (F) rats. Control, black circles; sham, open circles; ileectomy, black triangles; DJB, black squares; and jejunectomy, open squares. Data are shown as means  $\pm$  SE ( $n = 6$ –10 for each group). Significances are as follows. Insulin: \* $P < 0.005$  control vs. all other groups;  $\times P < 0.005$  control vs. ileectomy; + $P < 0.05$  control vs. DJB and ileectomy; # $P < 0.05$  control vs. sham, DJB, and jejunectomy; \*\* $P < 0.05$  control vs. all other groups. GIP Wistar rats: \* $P < 0.001$  ileectomy vs. sham, \*\* $P < 0.05$  DJB vs. sham; + $P < 0.05$  ileectomy vs. sham;  $\times P < 0.05$  ileectomy vs. control and sham. GIP GK rats: \* $P < 0.001$  ileectomy vs. all other groups; # $P < 0.01$  ileectomy vs. control, sham, and DJB.

tolerance after gastrointestinal surgery. In fact, the effects of sleeve gastrectomy on body weight and glucose tolerance are substantially preserved in GLP-1 receptor-deficient mice (41), and infusion of a GLP-1 receptor antagonist after RYGB does not reverse improvements in diabetes or glucose tolerance in humans (42). The results

of our study also show that DJB and jejunectomy improve whole-body  $S_I$  only in diabetic GK rats but not in normal Wistar rats, confirming earlier findings that DJB improves oral glucose tolerance in diabetic but not in glucose-tolerant animals (4). Taken together with the evidence that improved  $S_I$  after DJB and jejunectomy was



not associated with changes in GLP-1, GIP, or insulin, these findings support the hypothesis that bypass or resection of proximal segments of the small intestine might reduce or remove factor(s) that inhibit  $S_I$  (3,4). Furthermore, the fact that DJB and jejunectomy, but not ileal resection, improved  $S_I$  suggests that such putative factors may be located in or regulated by the proximal small intestine (duodenum and jejunum).

The question remains whether the primary site contributing to insulin resistance is the duodenum, jejunum, or both. The ability of jejunectomy to improve  $S_I$  in this study, as well as the reported improvement of diabetes after intestinal procedures that maintain duodenal passage of nutrients such as the jejuno-ileal bypass (20), would suggest a major role of the jejunum. In contrast, however, RYGB, DJB, and endoluminal duodenal sleeve bypass the entire duodenum and only a small segment of the proximal jejunum (12). The anatomic distinction between duodenum and jejunum is rather arbitrary, and most physiologic mechanisms of the intestinal mucosa show a typical proximal-to-distal (or vice versa) gradient. Hence, if the proximal small intestine of diabetic subjects produces or regulates factor(s) that inhibit  $S_I$  as suggested by this study, it is likely that this occurs according to a similar gradient rather than as a phenomenon restricted to an exact anatomic segment of the bowel. Accordingly, the control of diabetes after gastrointestinal bypass surgery may derive from the exclusion of both duodenum and jejunum, and procedures that bypass the entire length of the proximal small bowel may have more powerful effects on  $S_I$  than those with shorter lengths of intestinal bypass. Consistent with this hypothesis is the fact that bilio-pancreatic diversion (BPD), an operation that involves the greatest length of intestinal bypass (the duodenum and jejunum in their entirety) is associated with the best rates of long-term remission of diabetes (5,12). BPD results in major improvement of skeletal muscle  $S_I$ , mediated by changes in the expression of genes that regulate glucose and fatty acid metabolism in response to nutrient availability (43).

The anti-incretin theory (3,4,25,44) posits the existence of nutrients-stimulated mechanisms originating in or regulated by the proximal small bowel with the physiological role of preventing postprandial hypoglycemia from incretin-induced insulin secretion. Dysfunctional gastrointestinal physiology resulting in the disproportionate enhancement of anti-incretin mechanisms would result in insulin resistance and/or defects in insulin production predisposing to type 2 diabetes. Accordingly, a bypass of the proximal intestine (i.e., after RYGB, BPD, or DJB) could re-establish a physiologic balance between incretin and anti-incretin signals, thus restoring normal glycemic excursions. The anti-incretin theory predicts that intestinal bypass procedures (or resection of small bowel segments) would only improve glucose homeostasis in subjects with glucose

intolerance/diabetes but not in normotolerant individuals. This is consistent with the observation that DJB and jejunectomy in this study only improved  $S_I$  in diabetic rats but not in normal animals. Further experimental evidence in support of the anti-incretin theory derives from findings that proteins secreted by the duodenum/jejunum of diabetic *db/db* mice or insulin-resistant humans can induce insulin resistance both in vivo (in Swiss mice) and in vitro (myocytes cultures) (45).

We acknowledge several limitations of our study. First, our experiments did not investigate the exact molecular mechanisms behind the improvement of  $S_I$  after DJB or jejunectomy; our findings only allow us to exclude GLP-1 and GIP as factors. Furthermore, anatomic manipulations of the intestine induce adaptive intestinal responses that can influence energy homeostasis. Measuring  $S_I$  at a single time point, as we did in this study, may therefore depend on adaptive phenomena and not accurately reflect the steady state. As shown by Ljungmann et al. (46), however, rats that undergo resection of even 80% of the small bowel regain most of their body weight within 10 days from the operation, while most of the adaptive response of the residual small intestine occurs during the first week after surgery. Since we investigated glucose disposal 10 days after the operation, our findings should reflect a state after intestinal adaptation and weight recovery have already occurred. Also, our study investigated only the early effects of intestinal manipulations on insulin resistance and was not designed to assess potential clinical effectiveness of new procedures; hence, in no way do we propose jejunectomy as a possible operation in humans.

In conclusion,  $S_I$  improved in diabetic rats after both DJB and resection of the jejunum without changes in circulating incretin levels. These findings suggest a possible role of the proximal small bowel in the pathophysiology of insulin resistance and its improvement after gastrointestinal operations.

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**Author Contributions.** S.S. and A.B. were responsible for the design of the study, analysis of the data and mathematical modeling, writing of the manuscript, and results discussion. C.W.I.R. was responsible for writing of the manuscript, results discussion, and critical revision of the manuscript. F.R. was responsible for the experiments, writing of the manuscript, results discussion, and critical revision of the manuscript. G.M. was responsible for the design of the study, experiments, writing of the manuscript, and results discussion. G.M. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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